

WHAT IS CLAIMED IS:

Sub C4
1. A gram-negative bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 21, 25, 27, 29, 39, 41, 51, 53, 55, 57, 58, 60, 68, 72, 74, 76, 78, 80, 82, 84, 104, 108, 112, 116, 118, 120, 122, 124, 126, 128, and 130, or species homologs thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

2. The gram-negative bacteria of claim 1 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

3. The gram-negative bacteria of claim 1 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

4. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of all or part of said gene.

5. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

6. The gram-negative bacteria of claim 1 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

Sub C5
7. An attenuated *Pasteurellaceae* bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124,

126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

8. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

9. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

10. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in deletion of all or part of said gene.

11. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

12. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

13. The *Pasteurellaceae* bacteria of claim 7 selected from the group consisting of *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Haemophilus somnus*.

14. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

15. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

16. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of all or part of said gene.

17. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

18. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

19. The attenuated *Pasteurellaceae* bacteria of claim 13 that is a *P. multocida* bacteria.

20. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

21. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

22. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in deletion of all or part of said gene.

23. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least

about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

24. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

25. The attenuated *Pasteurellaceae* bacteria of claim 13 that is a *A. pleuropneumoniae* bacteria.

26. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

27. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

28. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in deletion of all or part of said gene.

29. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

30. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

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31. An immunogenic composition comprising the bacteria according to any one of claims 1 through 30.

32. A vaccine composition comprising the immunogenic composition according to claim 31 and a pharmaceutically acceptable carrier.

33. The vaccine composition according to claim 32 further comprising an adjuvant.

34. A method for producing a gram-negative bacteria mutant comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

35. A method for producing an attenuated *Pasteurellaceae* bacteria comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

36. A purified and isolated *Pasteurellaceae* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112,

114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, .

37. A purified and isolated *Pasteurellaceae* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 21, 25, 27, 29, 39, 41, 51, 53, 55, 57, 58, 60, 68, 72, 74, 76, 78, 80, 82, 84, 104, 108, 112, 116, 118, 120, 122, 124, 126, 128, and 130.

38. A purified and isolated polynucleotide encoding a *Pasteurellaceae* virulence gene product, or species homolog thereof, selected from the group consisting of:

- a) the polynucleotide according to claim 37,
- b) polynucleotides encoding a polypeptide encoded by the polynucleotide of (a), and
- c) polynucleotides that hybridize to the complement of the polynucleotides of (a) or (b) under moderate stringency conditions.

39. A purified and isolated *Pasteurellaceae* polynucleotide encoding a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165.

40. The polynucleotide of claim 39 which is a DNA.

41. A vector comprising the DNA of claim 40.

42. The vector of claim 41 that is an expression vector, wherein the DNA is operatively linked to an expression control DNA sequence.

43. A host cell stably transformed or transfected with the DNA of claim 40 in a manner allowing the expression of the encoded polypeptide in said host cell.

44. A method for producing a recombinant polypeptide comprising culturing the host cell of claim 43 in a nutrient medium and isolating the encoded polypeptide from said host cell or said nutrient medium.

45. A purified polypeptide produced by the method of claim 44.

46. A purified polypeptide comprising a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165.

47. An antibody that is specifically reactive with the polypeptide of claim 46.

48. The antibody of claim 47 that is a monoclonal antibody.

49. A method of using the monoclonal antibody of claim 39 for identifying a bacteria of claim 1, 7, 13, or 19 comprising the step of contacting an extract of bacteria with said monoclonal antibody and detecting the absence of binding of said monoclonal antibody.

50. A method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with expression or activity of gene products represented by the amino acid sequences set forth in any one of SEQ ID NOS: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123,

125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165 and identifying an agent that interferes with expression or activity of said gene products.

51. A method of identifying an anti-bacterial agent comprising the steps of:

- a) measuring expression or activity of a gene product as set out in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165
- b) contacting the gene product in (a) with a test compound
- c) measuring expression or activity of the gene product in the presence of the test compound; and
- d) identifying the test compound as an antibacterial agent when expression or activity of the gene product is decreased in the presence of the test compound as compared to expression or activity in the presence of the test compound.